Blood-clotting enzymology. The activation of factor X by factor IXa

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Blood-clotting enzymology. The activation of factor X by factor IX$_a$; the effect of phospholipid and of antihaemophilia factor VIII (F VIII) on the activation reaction.

The activation of bovine clotting factor X is mediated by the enzyme factor IX$_a$. Activation of factor X, *i.e.* the formation of the serine-protease X$_a$, is the result of the specific cleavage of a peptide bond in factor X. The rate of activation is strongly enhanced by the cofactor VIII$_a$ and by a phospholipid (PL) surface, onto which the protein adsorbs and where the reaction is thought to occur.

In order to get more insight into the mechanism of the activation reaction and the role of PL, Ca^{2+} and factor VIII, we have determined the kinetic constants of factor X activation and the effects of PL and factor VIII on these constants. Without PL and factor VIII$_a$, K_m for factor X is 272 μM and V is 6 mmol X_a mol^{-1} IX_a min^{-1}. With PL (10 μM) the K_m is lowered to 0.06 μM and V is unchanged. In the presence of PL and factor VIII$_a$, K_m is 0.02 μM and V is 111 mol X_a mol^{-1} IX_a min^{-1}. The factor X concentration in plasma is 0.16 μM, so we conclude that PL in plasma serves to lower the K to 0.06 μM, and that factor VIII stimulates factor X activation because it increases V of X_a formation at least 10 000 times.

We further investigated the effect of PL concentration on the kinetic parameters with factor VIII$_a$ left out. V for factor X_a formation does not change very much, but K_m for factor X increases from 0.06 μM at 10 μM PL to 1.35 μM at 260 μM PL. Earlier ROSING *et al.* (1980), studying the prothrombinase complex, found a similar relationship between PL concentration and the K_m for prothrombin.

This led to the hypothesis that it is the local substrate concentration at the phospholipid surface that determine the kinetics of its activation. An increased local concentration explains the dramatic decrease of K_m in the presence of PL. Further addition of PL lowers the substrate density at the PL surface with as consequence an increase of K_m.

The validity of this hypothesis can be tested if the binding parameters of substrate to PL are known. Recently, we found a new method to determine parameters of factor X binding to PL. This method is based on the observation that RVV-X (*i.e.* the factor-X-activating enzyme from Russell's viper venom) activates free factor X, but not factor X bound to PL. We found that PL vesicles consisting of equal amounts of phosphatidylserine and phosphatidylcholine (also used in our kinetic experiments) bind factor X with a dissociation constant (K_d) of 3.7×10^{-8} M. The concentration of factor-X-binding sites on these vesicles is 0.7 μM sites per 100 μM PL.

These data were used to convert K_m values from the kinetic experiments to K_m values expressed as factor X bound per PL surface area. The difference in K_m disap-

peared and one K_m value was found of 0.010 ± 0.003 μM factor X/μM PL for all PL concentrations. These results further support our hypothesis that the kinetics for X activation are governed by the density of the reactants bound to the PL surface.

Reference

ROSING, J., TANS, G., GOVERS-RIEMSLAG, J. W. P., ZWAAL, R. F. A. & HEMKER, H. C. (1980) *J. Biol. Chem.*, in press.